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Investigating the Potential of Chitosan-Nanocomposites as a Bio-Based Adsorbent for Sustainable Aflatoxin Eradication from Fish Feed

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Abstract

AFLATOXIN B1(AFB1), a hazardous mycotoxin frequently contaminates food and animal feed, represents a critical threat to global health. Chitosan (CS), a natural biopolymer derived from chitin, has emerged as a promising candidate for mycotoxin adsorption due to its unique properties. The study evaluated the aflatoxin adsorption potential of eight commercial products (P1-P8) under different conditions. P1 is Chitosan (CS), P2 is Nano Chitosan (NCS), P3 is Chitosan (CS) with mannan oligosaccharide (MOS), P4 is Chitosan (CS) with Bentonite (Bn), P5 is CS + MOS + Bn, P6 is NCS with MOS, P7 is NCS and Bn and P8 is NCS+ MOS+ Bn. The results showed substantial adsorption capacity across all evaluated products, with P8, a nano-chitosan formulation with both MOS and Bn, achieving the greatest efficiency (74.77%). P7 and P6 also demonstrated exceptional efficacy. P1 exhibited comparable adsorption to nano-chitosan (P2) at 67.70% and 68.82%, respectively. P5 and P4 displayed moderate adsorption capacities (68.10% and 61.30%), while P3 had a lower efficiency (59.30%). The study also examined the influence of environmental factors (pH and temperature) on the adsorption capacity of these products. P1 showed moderate adsorption capacity in fish feed matrices, ranging from 29.52% to 30.58%, depending on the P1/ fish feed ratio. P1's adsorption is sensitive to changes in pH and temperature, indicating the need for optimized treatment conditions. In conclusion, this research highlights the potential of chitosan and its derivatives as effective adsorbents for alleviating aflatoxin contamination in food and feed.

Keywords: Aflatoxin, Chitosan, mannan oligosaccharide, Bentonite, Fish Feed.

Introduction

Mycotoxins are secondary byproducts of toxigenic fungi, prevalent in both food and feed worldwide [1]. The detrimental impacts of mycotoxins on animals depend upon factors such as species, age, dosage, duration, as well as the nutritional and health status of the consuming organism [2]. The varied structural diversity of mycotoxins elicits a range of toxic effects in mammals, poultry, and fish [3]. Employing anti-mycotoxin feed additives presents an alternative and appealing approach to mitigate the risk of mycotoxicosis and reduce the transfer of mycotoxins from feed to animal products [4]. Primary strategies for mitigating mycotoxin contamination include implementing good agricultural practices in the field, such as crop rotation, soil cultivation, weed and insect control, and the judicious use of fungicides. Additionally, proper practices during harvest, transportation, and storage, including dry and cool conditions, are crucial [5].

The most prevalent mycotoxins in animal feed and human food are aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids [6]. Among these, aflatoxins (AF) are highly toxic substances produced by Aspergillus flavus and A. parasiticus. Aflatoxin B1 (AFB1) is particularly recognized for its hepatotoxicity among the aflatoxin group [7]. Aflatoxins (B1, B2, G1, and G2) are considered the most concerning group of mycotoxins on a global scale, owing to their toxicity [8]. Legislative limits and recommendations for these compounds have been established for feedstuff in Europe.

One effective approach to prevent or reduce the toxic effects of aflatoxin is the use of adsorbents. Adding selected adsorbents to aflatoxin-

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contaminated feeds can effectively bind aflatoxins during the process of the digestive system and allow the passing of mycotoxins harmlessly through the animal's system [9]. The animal feed industry has long employed feed additives to treat nutrient deficiencies, enhance product pigmentation, and improve the quality of feed pellets, and toxic substances and toxins are adsorbed [10]. Various substances, such as synthetic polymers (cholestyramine, polyvinyl-pyrrolidone, and derivatives), activated carbons, clay minerals (Bn, zeolite, etc.), organoclays, complex indigestible carbohydrates (cellulose, lignin, polysaccharides in MOS, and bacterial), have been investigated as potential mycotoxin adsorbing agents [11].

Furthermore, feed additives, antioxidants, sulfur-containing amino acids, vitamins, and trace minerals can be useful in mitigating the observed toxic effects in animals [12]. These substances are incorporated into the diet to lessen the distribution of mycotoxins to the circulation and target organs and their absorption from the gastrointestinal system [6]. Mycotoxin binders, which frequently go into feed are feed additives, work to reduce the toxicological potential of mycotoxins by adsorbing the toxins onto their surface [13]. One of the most cost-effective and promising strategies for minimizing animal and human exposure to mycotoxins involves the use of adsorbents in animal feed to decrease the absorption of mycotoxins in the gastrointestinal tract [14]. This approach has received significant attention over the past twenty years. Numerous chemical adsorbents, such as activated charcoal and aluminosilicates (such as zeolites, hydrated sodium calcium aluminosilicate, and clays), have undergone testing [15]. Chitosan (CS) possesses biodegradable and harmless properties. It readily dissolves in acidic conditions due to the presence of free amino groups on its polymer chains, which contribute to its positive charge [16].

Studies have demonstrated that CS and its derivatives can enhance fish growth performance, strengthen non-specific immunity, and exhibit antioxidant effects [17]. Chitosan (CS), a polysaccharide known for its biocompatible and biodegradable nature, serves as an excellent system of hydrophilic carriers [18]. CS has been utilized to improve fish digestibility, enhance the absorption of food components, and improve carnivorous fish's use of dietary carbohydrates [19]. Certain researchers have examined the effectiveness of CS's binding to different mycotoxins [20]. Conversely, several in vitro techniques have been created to evaluate the adsorbent capability of mycotoxin-sequestering compounds [21]. The delivery of DNA vaccines using CS has shown a much higher relative survival rate against bacterial and viral illnesses, ranging from 45% to 82% [22]. Drug delivery using CS nanoparticles has been used in research to support healthy gonadal development in aquaculture [23]. Ionotropic gelation, a procedure used to create CS nanoparticles, depends on the interaction of the positively and negatively charged amino groups of (CS) and pentasodium tripolyphosphate (TPP) to produce the nanoparticles. This method has been used to create (CS) nanoparticles that carry proteins and peptides [24].

The inhibitory mode of CS has been explained by three different methods. (CS) predominantly attacks the fungi's plasma membrane in the first mechanism. CS's positive charge makes it possible for it to interact with the phospholipids that make up the fungal membrane, which is negatively charged. This contact makes the membrane more permeable, which causes cellular contents to seep out and ultimately causes cell death [25]. According to the second mechanism, CS functions as a chelating agent by attaching to trace elements and blocking their availability for fungus to develop normally [26]. The third process suggests that CS can enter the fungal cell wall and attach DNA there. The generation of vital proteins and enzymes is impacted by this interaction because it prevents mRNA synthesis [27]. As there are Polysaccharides (mannans and glucans; MOS), proteins, and lipids found in MOS serve as a variety of adsorption sites, making them readily available for adsorption through multiple processes such as hydrogen bonding and ionic or hydrophobic interactions. It has been discovered that using MOS rather than complete yeast cells improves mycotoxin adsorption [28]. This paper explores the use of chitosan and its derivatives, including nano-chitosan, in adsorbing and detoxifying aflatoxins. It evaluates the adsorption efficiency of different CS formulations under various conditions, aiming to assess the potential of chitosan-based adsorbents as a solution for mitigating aflatoxin contamination in food and feed.

Material and Methods

Chitosan nanoparticles Preparation

Sigma-Aldrich (Germany) was the source of the high molecular weight CS (HMW) with a molecular weight (MW) of 310 kDa and a deacetylation degree of 90%. The ionotropic gelation process was employed to prepare (CS) nanoparticles [24]. 200 milliliters of 1% acetic acid solution contained two grams of chitosan powder dissolved in it. After that, 50 ml of NaCl solution (3 gr/lit) was added, and it was stirred for 30 minutes at 3000 rpm. To get rid of the insoluble chitosan, the solution was filtered through a Millipore 0.45 µm filter. After that, the sample was mixed dropwise with 20 ml of sodium tripolyphosphate (STPP) solution (3 gr/lit) while being stirred magnetically at 1000 rpm for five hours. For twenty minutes, the solution went through a centrifuge at 14,000 rpm. To remove of all remaining contaminants, the chitosan sediment was cleaned and

filtered three times with distilled water. Before being utilized for examination, the product was dried for 12 hours at 55 °C in an oven.

Zeta potential and particle size of the nano-chitosan

The zeta potential and particle size of the nano-chitosan composites were determined using Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS instrument (Malvern Instruments, UK). Samples were dispersed in deionized water, and measurements were taken at 25°C. The zeta potential was measured using the electrophoretic light scattering method, while the particle size was determined by measuring the diffusion of the particles using the dynamic light scattering technique.

Chitosan plus bentonite nanocomposite Synthesis

The bentonite used in the study was purchased from Sigma-Aldrich. The following is how the Chitosan plus bentonite (CS/Bn) was made: After dissolving the CS to the required weight ratio in 100 mL of 1% (v/v) acetic acid, the mixture was agitated for two hours to create an aqueous solution. A finite quantity, m, of Bn powder was dissolved in 50 mL of distilled water to create a Bn suspension, which was then sonicated for 15 minutes. After 24 hours of stirring, the CS solution was gradually added to the Bn suspension at 60 °C. To obtain modified 50%:50% (CS/Bn powder), the final nanocomposite was dried and rinsed with distilled water till pH =7. The morphology of the NCS, NCS+ Bn, NCS+ MOS, and NCS+ Bn+ MOS nanocomposites was observed using a Scanning Electron Microscope (SEM). The samples were mounted on conductive adhesive tape and sputter-coated with gold. Images were captured at various magnifications.

Chitosan plus mannan oligosaccharide nanocomposite Synthesis

A finite quantity, The MOS (YCW) was obtained from yeast industries, mannan oligosaccharide (MOS) powder was dissolved in 50 mL of distilled water to create a MOS suspension, which was then sonicated for 15 minutes. After 24 hours of stirring, the CS solution was gradually added to the MOS suspension at 60 °C. To obtain modified 50%:50% (CS/ MOS powder), the final nanocomposite was dried and rinsed with distilled water till pH = 7.

Chitosan plus bentonite plus mannan oligosaccharide nanocomposite Synthesis

Add mannan oligosaccharide to the mixture of Bn and CS. To encourage the MOS integration into the nanocomposite structure, stir at 60 °C to obtain modified the composite mixture. The composite mixture can be filtered to get rid of any large particles or contaminants once it has been thoroughly mixed. To obtain the chitosan/bentonite/mannan oligosaccharide nanocomposite in a solid state, the filtrate can be collected and dried using a method like air drying or freeze drying.

Aflatoxin Preparation

Calibration was done using 98% pure Aflatoxin B1 (AFB1) from Sigma (Germany) dissolved in a stock solution of 100µg/ml. The appropriate standard stock solutions for preparing calibration graphs were prepared in acetonitrile following the [29] technique. As earlier pointed out the public is ignorant of the existence of the bacterium [30]. Among the solvents used in the studies, were methanol, deionized water, and acetonitrile; all of them were HPLC-grade solvents provided by Merck Company. Pentasodium triphosphate (TPP) was analyzed by MSD and has a molecular weight of 367, while the Afla Test Immunoaffinity column (IAC) was purchased from VICAM Co. The sodium hydroxide (NaOH) used is 86 mmol and it has a molecular weight of 39. An Average molecular weight of 9971 g/mol were obtained from Merck kGaA, Germany while Acetate buffer and Phosphate-buffered saline were acquired from sigma-Aldrich Co. A standard curve for AFB1 was generated using an ELISA assay with a range of known AFB1 concentrations (0 - 100 µg/L). A minimum of five standards were used. Absorbance values at 450 nm were obtained for each standard using an ELISA plate reader (ELX 808, Bio-Tek Inst., USA). The absorbance values were then plotted against the corresponding AFB1 concentrations to create the standard curve.

Determination of ELISA for adsorbent sample

Sample preparation and analysis were crucial steps in the experiment. Fifty grams of ground samples were used for in vitro examination, creating a suspension with water and a magnetic stirrer. The suspension was pipetted into Falcon tubes, and acetate buffer and aflatoxin were added. Tubes were incubated at 37°C in an orbital shaker and then centrifuged to separate adsorbent materials. Supernatants were extracted and analyzed using an ELISA plate reader, with blanks and triplicates for each adsorbent. The percentage of aflatoxin adsorbed was calculated and compared to blank tubes. AFB1 analysis was conducted using a microtiter plate, following the Euro Proxima AFB1 test kit instructions. Absorbance was measured at 450 nm to determine aflatoxin concentration. These steps ensured an accurate evaluation of the adsorbent materials' effectiveness in aflatoxin removal. [31, 32].

pH and Temperature Impact

Chitosan Products were chosen to assess the impact of various incubation factors (temperature and pH) out of the peculiarities examined previously. Mögie D was positive for AFB1 with a level of 16. 65 ug/L. This then included Nano CS, CS, CS with Bentonite (Bn), CS with Yeast cell wall (MOS),

finally CS with MOS and Bn. Different pH levels (6. 1, and 8. 1) were subjected to sample pH to know the effect that it has. In the experimental case of AFB1, the concentrations of the adsorbents were selected to be 2 mg/mL. After expressing the protein once at 37 C, and once at 25 C for each pH level with moderate stirring (200 rpm) and centrifugation at 12,000 rpm for five minutes in a microcentrifuge, the sample was aspirated and extracted for assay by Elisa instrument. To investigate the effect of pH and temperature on adsorption efficiency, separate experiments were conducted using each chitosan product (NCS, CS, CS+ MOS+ Bn, and NCS+ MOS+ Bn) at two pH levels (6.1 and 8.1) and two temperatures (25°C and 37°C). A fixed concentration of AFB1 (16.65 µg/L) and adsorbent concentration (2 mg/mL) were used in each experiment. The adsorbent solutions were incubated with AFB1 for 2 hours at the designated pH and temperature with constant agitation (200 rpm). After incubation, the samples were centrifuged at 12,000 rpm for 5 minutes, and the supernatants were collected for analysis using the ELISA assay.

Determination of ELISA for adsorbents sample mixed with Fish feed sample

Fifty grams of the ground sample underwent extraction using 70% methanol, mixed forcefully on a magnetic stirrer, and filtered. The extract was then diluted with phosphate-buffered saline (PBS) for AFB1 analysis using a competitive ELISA commercial kit. AFB1 contamination was adjusted to 17 ug/L for binders mixed with fish feed and blank control. Samples were manually shaken and incubated overnight at room temperature. AFB1 analysis was conducted using a microtiter plate, following the kit instructions. The wells were filled with AFB1 detection solutions, test samples, conjugates, and antibody solution, incubated at 37°C, washed, and substrate solution was added. After incubation, the stop solution was added, and absorbance was measured at 450 nm using an ELISA plate reader. These steps ensured accurate AFB1 quantification in the samples [33]. The following equation was used to determine the mycotoxin adsorption % for each Sample: Adsorption (%) is calculated as (CB - CP) / CB × 100, where mycotoxin concentrations in the Supernatant (CP) and the Blank Control (CB) are both given in (ug/L) (Table 1).

Results

Adsorption Efficiency of Chitosan Products without Fish Feed Samples

The adsorption efficiencies of eight different chitosan products, described in Table 2, were evaluated under varying conditions. In vitro studies were conducted to assess the adsorption efficiency of various chitosan-based products against aflatoxin B1 (AFB1). The initial AFB1 concentration was 16.65 µg/L, as determined by an ELISA assay using a standard curve (Fig. 1). The results (Table 3) demonstrated a significant reduction in AFB1 content across all evaluated chitosan products. P1 and P2 exhibited the highest adsorption efficiencies, with percentages of 67.747% and 68.828%, respectively. This indicates that both chitosan alone and in its nano form can effectively bind and remove aflatoxins from the solution. Combinations of CS with MOS and Bn (P5) resulted in moderate adsorption percentages, with CS plus MOS (P3) achieving 59.339% and CS plus Bn (P4) reaching 61.321%. These results suggest that the addition of MOS or Bn to CS formulations may have a positive, but limited, impact on adsorption efficiency. Interestingly, (P5) displayed an adsorption percentage of 68.108%, similar to the performance of CS alone, implying that equal amounts of CS, MOS, and Bn can act synergistically to enhance adsorption capacity. Nano Chitosan (NCS) based combinations also showed promising adsorption percentages, with NCS+MOS (P6) achieving 63.24% and NCS+ Bn (P7) reaching 68.64%. The highest adsorption percentage (74.77%) was observed for the combination of NCS+ MOS+ Bn (P8), suggesting that incorporating nanoparticles into CS formulations, alongside MOS and Bn, can further enhance aflatoxin adsorption performance.

Adsorption Efficiency in Fish Feed Samples

To evaluate the adsorption efficiency of chitosan products in a realistic context, the study examined the adsorption of AFB1 when chitosan was mixed with a fish feed sample. The fish feed sample was spiked with AFB1 to achieve a concentration of 17 µg/L. Two chitosan products were evaluated PR1 and PR2 (Table 1). The results (Table 3) revealed that CS mixed with fish feed exhibited a moderate adsorption percentage of 30.58%, while NCS mixed with fish feed showed a lower adsorption percentage of 20.47%. This indicates that the presence of fish feed components can influence the adsorption capacity of CS and its derivatives.

Effect of Incubation Conditions on Adsorption Efficiency

To investigate the influence of pH and temperature on the adsorption efficiency of different chitosan-based products, further experiments were conducted using NCS, CS, CS+ MOS+ Bn, and NCS+ MOS+ Bn. The AFB1 concentration was maintained at 16.65 µg/L, and the adsorbent concentration was 2 mg/ml. The results (Table 4) demonstrated that the adsorption efficiency was significantly higher at pH 4.1 compared to pH 6.1 and pH 8.1, suggesting that slightly acidic conditions are more favorable for AFB1 adsorption by chitosan-based products. The incubation temperature also influenced the adsorption capacity, with higher efficiencies observed at 37°C compared to 25°C at both pH levels. This indicates that higher temperatures

facilitate AFB1 adsorption by the tested chitosan-based products. Overall, the NCS+ MOS+ Bn combination consistently demonstrated the highest adsorption efficiency across various pH levels and incubation conditions, suggesting a synergistic effect between chitosan, MOS, and Bn in enhancing the adsorption capacity of AFB1.

Characterization of Chitosan Products

To understand the physicochemical properties of chitosan products, Fourier Transform Infrared (FTIR) analysis and Scanning Electron Microscopy (SEM) imaging were conducted. FTIR spectroscopy analysis (Figure 2, Table 5) revealed the presence of characteristic functional groups in chitosan, bentonite, MOS, and their composites. The identified functional groups were responsible for the adsorption of AFB1 through various interactions, including hydrogen bonding, ionic, and hydrophobic interactions. SEM imaging (Figure 3) revealed the morphology of the CS+ Bn+ MOS nanocomposite. The image showed a porous structure, which can contribute to increased surface area and enhanced adsorption capacity.

Zeta Potential and Particle Size Analysis

The zeta potential and particle size of the nano CS composites were measured using dynamic light scattering (Table 6). NCS exhibited a positive zeta potential of +43.12 mV, suggesting its ability to interact with negatively charged surfaces. The addition of MOS and Bn resulted in lower zeta potentials, which may be attributed to the presence of negatively charged groups on these materials. The particle size analysis revealed a wide range of particle sizes for the NCS composites, with mean sizes ranging from 73.15 nm to 125.32 nm. The results from the characterization studies provided valuable insights into the physicochemical properties of the chitosan products, which are important factors in understanding their adsorption behavior.

Discussion

This research paper presents a promising investigation into the potential of chitosan and its derivatives as adsorbents for aflatoxin B1 (AFB1), a significant mycotoxin contaminant in food and feed. The study demonstrates the effectiveness of different chitosan-based formulations in removing AFB1, highlighting the importance of chitosan nanoparticles and the synergistic effects of combining chitosan with mannan oligosaccharide (MOS) and bentonite (Bn).

The findings of this study demonstrate the promising potential of chitosan (CS) and its derivatives as effective adsorbents for mitigating aflatoxin B1 (AFB1) contamination in food and feed. The results highlight the significant adsorption capacity of various chitosan products, with the combination of nano-chitosan (NCS), mannan oligosaccharide (MOS), and bentonite (Bn) (P8) exhibiting the highest efficiency, reaching 74.77% AFB1 removal. The superior adsorption capacity of P8 can be attributed to the synergistic effects of the individual components. Nano-chitosan (NCS), with its high surface area and positive charge, effectively binds to the negatively charged AFB1 molecules [34]. Mannan oligosaccharide (MOS), a complex polysaccharide with various adsorption sites, further enhances the binding capacity through multiple mechanisms like hydrogen bonding and hydrophobic interactions [35]. Bentonite (Bn), a clay mineral, provides additional binding sites and contributes to the overall adsorption process [36]. The study also highlights the significant role of chitosan nanoparticles (NCS) in enhancing adsorption efficiency. While chitosan alone (P1) exhibited good AFB1 removal (67.747%), NCS (P2) displayed a slightly higher efficiency (68.828%), demonstrating the potential of reducing particle size to increase surface area and improve adsorption. The influence of environmental factors on adsorption efficiency was investigated, demonstrating that slightly acidic conditions (pH 4.1) are more favorable for AFB1 adsorption by chitosan-based products. This observation is consistent with previous research highlighting the importance of pH in mycotoxin adsorption [37-39].

The increased adsorption at higher temperatures (37°C) suggests that the binding process is facilitated by the increased molecular movement and interactions at elevated temperatures. However, the study also revealed that the adsorption capacity of chitosan is influenced by the presence of feed components. This suggests that the complex matrix of fish feed ingredients can interfere with the adsorption process. The findings of this study have significant implications for the development of novel strategies to mitigate aflatoxin contamination in food and feed. Chitosan-based adsorbents, particularly those incorporating nano-chitosan, MOS, and Bn, show promise for effective AFB1 removal.

There is a significant impact of environmental factors, specifically pH and temperature, on the adsorption efficiency of chitosan-based products against aflatoxin B1 (AFB1). The findings demonstrate that both pH and temperature significantly influence the adsorption process, highlighting the importance of optimizing conditions for maximizing AFB1 removal. The influence of temperature on adsorption efficiency was investigated. The results demonstrate that higher temperatures (37°C) lead to increased adsorption compared to lower temperatures (25°C). This suggests that elevated temperatures facilitate molecular movement and interactions, promoting stronger binding between chitosan and AFB1 [40]. The consistent observation of the highest adsorption efficiency for the NCS+ MOS+ Bn combination

across various pH levels and temperatures suggests a synergistic effect between chitosan, MOS, and Bn. The combination likely provides a greater diversity of binding sites and mechanisms, enhancing adsorption capacity. The findings of this study contribute significantly to the understanding of the factors influencing the adsorption efficiency of chitosan-based adsorbents, paving the way for the development of more effective solutions for managing aflatoxin contamination.

Chitosan and its derivatives demonstrate promising results in controlled in vitro settings, their performance can be significantly affected by the presence of various components in fish feed samples. The study found that CS mixed with fish feed (PR1) exhibited a moderate adsorption percentage (30.58%), while NCS mixed with fish feed (PR2) showed a lower adsorption percentage (20.47%). These findings are notably lower than the adsorption efficiencies observed in controlled in vitro settings, highlighting the impact of the complex fish feed matrix on the adsorption process. Several factors likely contribute to the reduced adsorption efficiency in fish feed, The first Competition for Binding Sites: Fish feed components, including proteins, carbohydrates, and lipids, may compete with AFB1 for binding sites on the chitosan surface, reducing the amount of AFB1 adsorbed [41]. The second Matrix Effects: The presence of other constituents in the fish feed matrix may interfere with the adsorption process, altering the accessibility of AFB1 to the chitosan adsorbent [42]. The third Physical Accessibility: The physical structure of the fish feed matrix may limit the accessibility of AFB1 to the chitosan adsorbent, hindering effective binding [43]. While the results indicate a potential limitation of chitosan-based adsorbents in complex fish feed matrices, they also emphasize the importance of ongoing research to develop more effective solutions for managing aflatoxin contamination in animal feed.

The characterization studies using Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) provide valuable insights into the physicochemical properties of chitosan (CS), bentonite (Bn), mannan oligosaccharide (MOS), and their composites, which underpin their adsorption capabilities. FTIR analysis revealed the presence of characteristic functional groups in each material, including the stretching vibrations of O-H, the bending vibrations of amide II -NH2, and the stretching of C-N and C=O bonds. These functional groups play a crucial role in the adsorption process, facilitating interactions like hydrogen bonding, ionic interactions, and hydrophobic interactions between the adsorbent materials and AFB1 as mentioned in [44-46]. The SEM image of the CS+ Bn+ MOS nanocomposite revealed a porous structure, suggesting a high surface area, which further contributes to its enhanced adsorption capacity [47, 48]. The porous morphology allows for greater contact between the adsorbent material and AFB1, facilitating efficient binding and removal [49]. The identified functional groups and the porous structure of the composite material collectively contribute to the observed high adsorption efficiency of this chitosan-based formulation [50].

The characterization of nano-chitosan (NCS) composites through zeta potential and particle size analysis provides valuable insights into their physicochemical properties and potential impact on aflatoxin B1 (AFB1) adsorption. The positive zeta potential of NCS (+43.12 mV) suggests its ability to interact electrostatically with negatively charged surfaces, such as AFB1 molecules. This electrostatic attraction is likely a key factor in the observed adsorption efficiency of NCS [40]. The addition of bentonite (Bn) and mannan oligosaccharide (MOS) to the NCS composite resulted in lower zeta potentials, which may be attributed to the presence of negatively charged groups on these materials [51, 52]. This reduction in zeta potential could influence the adsorption process, potentially leading to a more complex interplay of electrostatic and other interactions. The particle size analysis reveals a range of particle sizes for the NCS composites, with mean sizes varying from 73.15 nm to 125.32 nm. The variation in particle size across different formulations is noteworthy. A smaller particle size generally indicates a larger surface area, which can enhance adsorption capacity by providing more binding sites for AFB1 [53, 54]. However, the effect of particle size on adsorption efficiency in these composites needs further investigation. The specific combination of materials and their particle size distribution may influence the overall adsorption capacity. This research contributes significantly to the field of mycotoxin management and food safety. By addressing these research gaps, chitosan-based adsorbents hold promise as a valuable tool for reducing aflatoxin contamination in food and feed, contributing to improved food safety and animal health.

Conclusion

The study demonstrates the effectiveness of chitosan (CS) and its derivatives, particularly nano CS, in adsorbing and detoxifying aflatoxins. CS and nano CS exhibited significant adsorption capabilities, with the highest adsorption percentage of 74.77% observed for the combination of nano CS, MOS, and Bn at a ratio of 1:1:1. The adsorption efficiency is influenced by pH level, with slightly acidic conditions favoring higher adsorption. The study highlights the potential of CS-based adsorbents in improving food safety and reducing health risks associated with aflatoxin contamination. Additionally, the use of CS for aflatoxin

detoxification is economically viable, making it a promising solution for the food and feed industries.

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Conflicts of interest

There are no conflicts to declare. The authors declared no competing interests.

Author Contributions

All authors contributed equally to this work (conception, acquisition, samples analysis, statistical analysis, data interpretation, manuscript drafting, and manuscript revision).

TABLE 1. Chitosan Products Mixed with Feed for Adsorption Efficiency Analysis.

Product	Composition
PR1	(5 g) of (CS) mixed with feed (45 g)
PR2	(5 g) of (NCS) mixed with feed (45 g)

The table lists the compositions of the two chitosan products (CS and NCS) that were mixed with feed for evaluating their aflatoxin adsorption efficiency in a realistic context.

TABLE 2. List of Chitosan Products Evaluated in the Adsorption Study.

Product	Composition	Amount 100mg
P1	CS	1
P2	NCS	1
P3	CS+ MOS	(50%:50%)
P4	CS+ Bn	(50%:50%)
P5	CS+ MOS+ Bn	(1:1:1)
P6	NCS+ MOS	(50%:50%)
P7	NCS+ Bn	(50%:50%)
P8	NCS+ MOS+ Bn	(1:1:1)

The table provides the compositions of the eight different chitosan products tested, including chitosan alone (CS), nano-chitosan (NCS), and various combinations with mannan oligosaccharide (MOS) and bentonite (Bn).

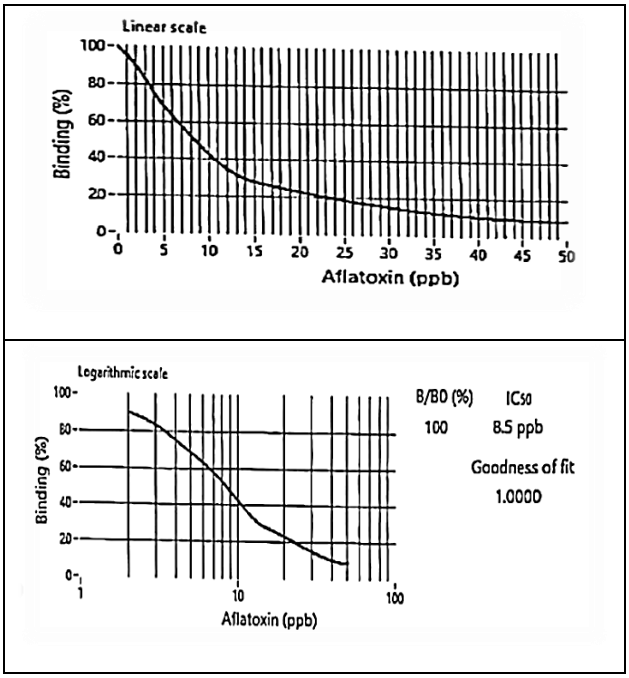

Fig.1. Standard Curve for Aflatoxin B1 (AFB1) Determination using ELISA Assay.

TABLE 3. Adsorption Efficiencies of Different Chitosan Products at pH 4.1.

Adsorbents	Product abbreviation	AFB1 Content (ug/kg)	Adsorption (%)
Control Blank	CB1	16.65	0
CS (100%)	P1	5.37	67.747
NCS (100%)	P2	5.19	68.828
CS+ MOS (50%:50%)	P3	6.77	59.339
CS+ Bn (50%:50%)	P4	6.44	61.321
CS+ MOS + Bn (1:1:1)	P5	5.31	68.108
NCS+ MOS (50%:50%)	P6	6.12	63.24
NCS+ Bn (50%:50%)	P7	5.22	68.64
NCS+ MOS + Bn (1:1:1)	P8	4.2	74.77
Control Blank for feed sample	CB2	17	0
CS Mixed with Feed	PR1	11.80	30.58
NCS Mixed with Feed	PR2	13.52	20.47

The table presents the percentage adsorption of aflatoxin B1 (AFB1) by various chitosan products, including chitosan alone (CS), nano-chitosan (NCS), and combinations with mannan oligosaccharide (MOS) and bentonite (Bn). The adsorption efficiencies were measured at a pH of 4.1.

TABLE 4. Adsorption Efficiencies of Selected Chitosan Products at pH 6.1 and 8.1 at 37°C.

Adsorbents	Temperature	Adsorption efficiency (%) at pH 6.1	Adsorption efficiency (%) at pH 8.1
CS	37	64.3	60.7
	25	61.9	58.7
NCS	37	66.9	63.2
	25	65	61.9
CS+ MOS + Bn	37	66.2	62.1
	25	64.2	61.3
NCS+ MOS + Bn	37	71.4	67.9
	25	68.7	66.1

The table displays the percentage of AFB1 adsorption by chitosan (CS), nano-chitosan (NCS), and a combination of CS, MOS, and Bn at pH levels of 6.1 and 8.1, at temperatures of 37°C and 25°C.

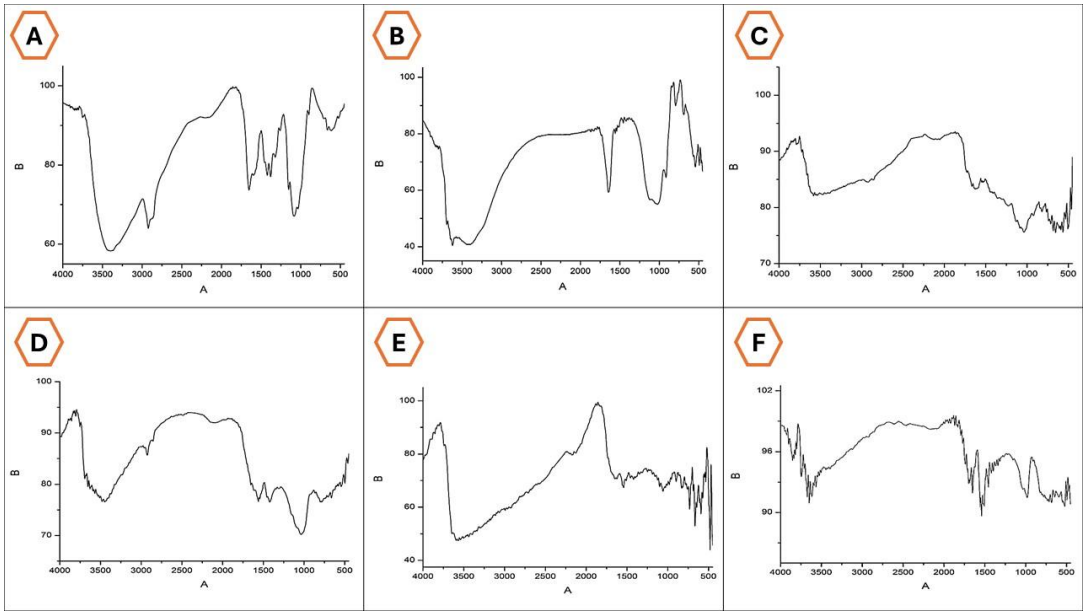

Fig. 2. Fourier Transform Infrared (FTIR) spectrum analysis for Chitosan "CS" (A), Bentonite "Bn" (B), mannan oligosaccharide "MOS" (C), Chitosan "CS" plus Bentonite "Bn" (D), Chitosan "CS" plus mannan oligosaccharide "MOS" (E) and Chitosan "CS" plus Bentonite "Bn" plus mannan oligosaccharide "MOS" (F).

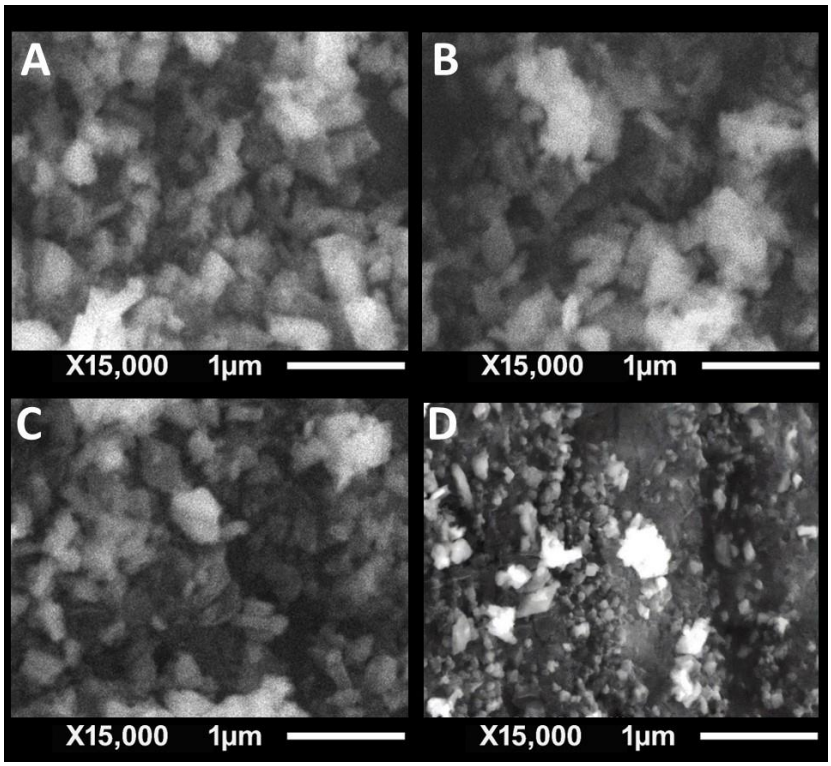

Fig. 3. Shows the Scanning Electron Microscopy (SEM) images of four different samples: Nano Chitosan (A), Nano Chitosan + Mannan Oligosaccharide (B), Nano Chitosan + Bentonite (C), and Nano Chitosan + Bentonite + Mannan Oligosaccharide Nanocomposite. The SEM images display the morphology of the nanocomposite, emphasizing its porous structure.

TABLE 5. Functional Groups Identified in Chitosan (CS), Bentonite (Bn), Mannan Oligosaccharide (MOS), and Their Composites.

Functional groups	Wavenumbers					
	NCS	Bn	MOS	NCS+Bn	NCS+MOS	NCS+BN+MOS
Stretching of O – H	3430	3410	3590	3450 3420	3598	3625
Amide II -NH2 bending	1720	1700	1690	1600	1720	1710
Stretching of C-N	1410	1460	1407	1503	1410	1445
C = O in the amide group	1649	1655	1649	1598	1649	1549
Amide III vibrations, N-H stretching vibrations, C-N bond stretching vibrations	1389	-	1375	-	-	-
stretching of C-O	1080	1050	1059	1038	1080	1000
Antisymmetric C-O stretching	835	-	837	-	835	-
Si – O	-	1050	-	1038		1000
Symmetric and Asymmetric C – H	2925	-	2933	2960	2932	2910
Asymmetric Stretching O - Si – O	-	840	-	808	-	901
(Deformation and Bending), Si – O	-	730	-	680	-	715
Asymmetric Bending O - Si – O	-	545	-	540	-	512

This table shows the characteristic functional groups present in each of the materials, based on their FTIR spectra analysis. The presence of these functional groups is important for understanding the mechanisms of adsorption.

TABLE 6. Physical Characteristics of Nano-Chitosan (NCS) Composites.

Ingredient	Particle size range (nm)	Particle size mean (nm)	Charge (zeta potential, mV)
NCS	43.65-409.69	73.15	+ 43.12
Bn	ND	ND	- 22.48
MOS	ND	ND	- 19.94
NCS+MOS	54.45-593.81	111.85	+ 23.61
NCS+ Bn	69.23-613.52	106.73	+ 20.76
NCS+ Bn+ MOS	66.67-682.68	125.32	+ 13.35

The table presents the particle size range, mean particle size, and zeta potential of different nano-chitosan composites, including nano-chitosan (NCS), nano-chitosan with bentonite (NCS+ Bn), nano-chitosan with mannan oligosaccharide (NCS+ MOS), and nano-chitosan with bentonite and mannan oligosaccharide (NCS+ Bn+ MOS).

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دراسة إمكانات مركبات الكيتوزان النانوية كمواد ماصة حيوية للقضاء على الأفلاتوكسين بشكل مستدام من أعلاف الأسماك

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الملخص

يُهدد الأفلاتوكسين ب 1(AFB1) ، وهو سم خطير، صحة الإنسان والحيوان بشكل متكرر من خلال تلوثه للغذاء وعلف الحيوانات. ولحسن الحظ، ظهر الكيتوزان (CS) كأمل واعد للتخلص من هذه السموم، وذلك بفضل خصائصه الفريدة كمرشح طبيعي مشتق من الكيتين. قامت هذه الدراسة بتقييم قدرة امتصاص الأفلاتوكسين لثمانية منتجات تجارية-P1) (P8في ظروف مختلفة P1 .هو الكيتوزان(CS) ، P2هو الكيتوزان النانوي(NCS) ، P3هو الكيتوزان (CS) مع أوليجو سكر المانان(MOS) ، P4هو الكيتوزان (CS) مع البنتونيت(Bn) ، P5 هوBn + MOS + CS ، P6هو NCS مع MOS ، P7هو NCS و Bn و P8 هو .Bn + MOS + NCS أظهرت الدراسة نتائج مبهرة، حيث أبدت جميع المنتجات قدرة عالية على امتصاص الأفلاتوكسين. وبرزP8 ، وهو تركيبة نانوية من الكيتوزان مع كل من MOS وBn، كأفضل منتج بفعالية امتصاص وصلت إلى 74.77%. كما أظهر كل من P7 و P6فعالية استثنائية. بينما أظهر P1 قدرة امتصاص مماثلة للكيتوزان النانوي (P2) بنسبة 67.70% و68.82% على التوالي. أما P5 و P4فقد أظهرا قدرات امتصاص معتدلة (68.10% و61.30%)، بينما كانت كفاءة P3 أقل (59.30%). تابعت الدراسة تأثير العوامل البيئية، مثل درجة الحموضة ودرجة الحرارة، على قدرة امتصاص الكيتوزان للأفلاتوكسين. وأظهرت النتائج أن قدرة P1 على الامتصاص في مصفوفات علف الأسماك كانت معتدلة، وتراوحت بين 29.52% و30.58%، اعتمادًا على نسبة / P1 علف الأسماك. كما أظهرت الدراسة أن امتصاص P1 حساس للتغيرات في درجة الحموضة ودرجة الحرارة، مما يدل على ضرورة تحسين ظروف المعالجة لتحقيق أقصى قدر من الكفاءة. ختامًا، يسلط هذا البحث الضوء على إمكانات الكيتوزان ومشتقاته كممتصات فعالة للتخفيف من تلوث الأفلاتوكسين في الغذاء والعلف.

الكلمات الدالة: الأفلاتوكسين، الكيتوزان، أوليجو سكر المانان، البنتونيت، علف الأسماك.